ApoScreen® Annexin V Apoptosis Kit-FITC

Description

ApoScreen® Annexin V Apoptosis Kit-FITC (SB Cat. No. 10010-02) employs a fluorescein-conjugated Annexin V (Annexin V-FITC) in concert with propidium iodide (PI) to evaluate subpopulations of cells undergoing apoptosis. During the early stages of apoptosis, cells begin to display phosphatidylserine (PS) on the outer cell membrane where it is readily detectable by staining the cells with Annexin V-FITC. As the plasma membrane becomes increasingly permeable during the later stages of apoptosis, PI can readily move across the cell membrane and bind to cellular DNA, providing a means for identifying those cells that have lost membrane integrity through mechanisms including necrosis. When cells are double stained with Annexin V-FITC and PI, three different cell populations may be observed - (i) live cells that do not stain with either Annexin V-FITC or PI; (ii) necrotic cells that stain with both reagents; and (iii) apoptotic cells that stain with Annexin V-FITC only. Analysis may be performed on any flow cytometer equipped with a single laser using excitation at 488 nm. Alternatively, the cells can be examined with a fluorescence microscope equipped with filters for discrimination of FITC and rhodamine/PI.

Applications

FC – Quality tested 1-7, 9-34
ICC – Reported in literature 6,8,35

Kit Components & Storage and Handling

- Annexin V-FITC is supplied as 100 tests in 1.0 mL of Annexin V Binding Buffer. Store at 2-8°C.
- Propidium Iodide (PI) is supplied as 100 tests in 1.0 mL of Annexin V Binding Buffer. Store at 2-8°C.
- Annexin V Binding Buffer 10X is supplied as 7 x 1.5 mL. Store at 2-8°C.
- Protect FITC conjugated and PI reagents from light. Reagents are stable for the period shown on the label if stored as directed.

Working Dilutions

**Flow Cytometry**
- Annexin V-FITC 10 μL/10⁶ cells
- Propidium Iodide (PI) 10 μL/10⁶ cells
- Annexin Binding Buffer 10X Dilute 1 part 10X buffer with 9 parts dH₂O just prior to use

For flow cytometry, the suggested use of these reagents is in a final volume of 100 μL of Annexin Binding Buffer 1X

Other Applications

- Since applications vary, you should determine the optimum working dilution for the product that is appropriate for your specific need.

Suggested Staining Protocol

- Wash cells twice in cold PBS
- Resuspend cells in cold 1X Annexin Binding Buffer to a concentration of 1 x 10⁶ cells/mL
- Add 100 μL of cell suspension (10⁶ cells) to 5 mL polystyrene round bottom tubes
- Add 10 μL of conjugated Annexin V-FITC
- Gently vortex each tube and incubate for 15 minutes at 2-8°C and protected from light
- Without washing, add ~380 μL of cold 1X Annexin Binding Buffer to each tube
- Add 10 μL of Propidium Iodide (PI)
- Analyze by flow cytometry immediately

For Research Use Only. Not for Diagnostic or Therapeutic Use.
References


Warning
Some reagents contain sodium azide. Please refer to product specific (M)SDS.